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The effect of feeding inorganic and organic selenium sources on the hematological blood parameters, reproduction and health of dairy cows in the transition period

Mohsen Khalili[1], Mohammad Chamani[1*], Hamid Amanlou[2], Ali Nikkhah[1], Ali Asghar Sadeghi[1], Farhad Kamali Dehkordi[2],Mohammad Rafiei[3] and Vahid Shirani[4]

[1]Department of Animal Science, Science and Research Branch, Islamic Azad University, 1477893855, Tehran, Iran. [2]Department of Animal Science, University of Zanjan, Zanjan 45371-38791, Iran. [3]Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. [4]Department of Animal Science, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran. *Author for correspondence. E-mail: m.chamani@srbiau.ac.ir

ABSTRACT. The purpose of this study was to investigate the effects of four types of diets containing different forms of selenium on the hematological blood parameters, reproduction and health of dairy cattle during the transition period. Twenty-four close-up dry cows with a mean of 259 ± 1days of pregnancy and expected 21 ± 1days prior to parturition were selected. The cows were fed four diets: 1) basal diet without selenium supplementation (C); 2) basal diet plus 0.5ppm selenium in the form of sodium selenite (Se-S) 3) basal diet plus 0.5ppm selenium in the form of selenium yeast (Se-Y); 4) basal diet plus 0.5ppm selenium in the form of selenomethionine (Se-M). Blood samples for measuring hematological blood parameters (including lymphocyte, red blood cells, hemoglobin, and hematocrit, MCV, MCH, MCHC and RDWc) were taken at 21 and 10 days before delivery, delivery date, 10 and 21 days after delivery. There was no significant difference in hematological parameters before and after delivery in experimental and control groups. However, in the prepartum period, MCH tended to increase significantly in selenium methionine treatment ($p<0.05$). Reproductive parameters (including distance to first estrus, insemination indexes and number of open days) and health parameters (including rectum temperature and vaginal content score) have been improved by selenium organic supplements. The results of this experiment showed that feeding organic selenium supplementation in multiparous dairy cow's diet may improve their health and reproduction.

Keywords: selenium; hematological blood parameters; reproduction; health; dairy cows.

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Introduction

Selenium is an important trace mineral and selenium deficiency has a negative effect on the health of humans and animals (Alfthan et al., 2015; Hatfield, Tsuji, Carlson, & Gladyshev, 2014). One of the important functions of selenium can be its antioxidant role against free radicals containing particles to prevent cancer and its role in the functions of glutathione peroxidase and other reductase (Albanes et al., 2014; Shaheen, Rinklebe, Frohne, White, & DeLaune, 2014; Speckmann & Grune, 2015). Due to the increasing age of the population and the increase in the number of people involved with cancer and other immune-related diseases, there is a demand for selenium-rich milk. Therefore, increasing selenium intake by selenium-rich foods, such as rice, garlic, onions and broccoli, meat, milk and eggs have been considered as one of the interesting topics (Calamari, Petrera, & Bertin, 2010; Lin, 2014; Pophaly, Singh, Kumar, Tomar, & Singh, 2014; Yasin, El-Mehdawi, Anwar, Pilon-Smits, & Faisal, 2015). Foods can be supplemented with selenium in two forms, an inorganic form such as sodium selenite and sodium selenate or an organic form such as selenium yeast and selenium methionine. The metabolism of these two forms is different in animals. Organic selenium is absorbed through active transmission in the small intestine and in the synthesis of proteins; it is stored instead of methionine in tissues. It provides a source of selenium in the organs and tissues (Schrauzer, 2003). However, inorganic selenium is absorbed through passive transmission and is retained in small amounts in the body's stores, and a large amount is excreted through feces and urine. Thus, in recent years, the shift in supplements from the form of inorganic selenium (sodium selenite) to organic forms (selenium yeast and selenium methionine) is further emphasized.

Selenium deficiency presents a factor favoring the appearance of perinatal metritis and retention of placenta in dairy cattle. In addition, selenium deficiency can cause a malfunction of the testosterone and

spermatozoon synthesis, which causes infertility in males. Selenium is known to influence the gross and histological morphology of the testis. Selenium deficiency is often characterized by reduced spermatozoon motility due to the fragility of its intermediate piece. Some selenoproteins were localized in the testes as selenophosphate synthase-2 (SPS-2) and the mitochondrial capsule selenoprotein (MCSeP). An increase of the selenium content in the testes of cattle was reported during the supplementation with selenium enriched cereals, mineral selenium (selenite) and organic selenium (yeast). The increase in fertility when adding selenium can be attributed to the reduction in embryonic death in the first month of gestation (Mehdi & Dufrasne, 2016).

According to Hall et al. (2014) feeding selenium-replete cows during late gestation a supranutritional selenium yeast supplement improves antioxidant status and immune responses after calving. There is a relationship between selenium content in the diet and mastitis frequency in cows, knowing that the phagocytic activity of neutrophils is the primary defense mechanism against mastitis. Selenium affects the innate and the adaptive immune responses of the mammary gland through humoral and cellular activities (Mehdi & Dufrasne, 2016). According to Finch and Turner (1996) several researchers have demonstrated a significant reduction in the incidence of mastitis in dairy cows after they were supplemented with selenium and/or vitamin E. The aim of this study is to investigate the effects of selenomethionine, selenium yeast and sodium selenium supplements on hematological blood parameters, reproduction and health of dairy cows in the transition period.

Material and methods

Animal and dietary treatment

The present study was conducted in the FKA animal husbandry and Agriculture Company in the province of Isfahan Iran. The study began in the early May and lasted to late July, 2016. Twenty-four multiparous dairy Holstein cows (parity 3) with an average weight of 791 ± 50 kg were selected 21 days before the expected parturition. The previous production of their milk did not differ significantly ($p = 0.88$) and they were randomly assigned to four groups and placed in separate 3 × 3 m booths with separate drinkers and feeders. The diets were formulated based on NRC (2001) recommendation for a dry cow (close up period) and fresh lactating dairy cow (Table 1). The diets included: 1) basal diet without selenium supplementation (C); 2) basal diet plus 0.5 ppm Se in the form of sodium selenite (Se-S) 3) basal diet plus 0.5 ppm Se in the form of selenium yeast (Se-Y); 4) basal diet plus 0.5 ppm Se in the form of selenomethionine (Se-M). The Na2SeO3 (MerkCo., Germany) was used as the inorganic source of Se. The organic sources of Se were in the form of Se-enriched yeast (Biorigin., Brazil – Selemax 2000 ppm) and the form of Se-methionine (Arkop., Poland – Amino Selstar 2000 ppm). Selenium methionine, selenium yeast and sodium selenite supplements were used in addition to the control diet. The mineral supplement of the control diet was without selenium and all three forms of selenium were added to the total mixed ration as topdress. Before the morning feeding, daily feed residuals were collected, weighed and 300 g of them were frozen in nylon bags for the analysis. The amounts of Se concentrations in diets were measured by an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) device (using a Varian Model SpectrAA 220 atomic absorption spectrometer, Mulgrave, Victoria, Australia) (Table 1).

Sampling and data collection

Samples of total mixed ration (TMR) and orts were weekly taken for dry matter (DM) measurement, and were dried at 60° C for 48 h, and then composited by treatment. Dried pooled samples of TMR diets and refusal were ground through a 1-mm screen in a Wiley Mill and analyzed for analytical DM Association Official Analytical Chemist [AOAC], 2005), method 930.15), crude protein (CP) by the Kjeldahl method (AOAC, 2005), method 984.13), ether extract by the Soxhlet extraction method with diethyl ether (AOAC, 2005), method 920.39), ash (ignition at 600° C for 2 h; (AOAC, 2005), method 942.05), and ADF by the cetyl-trimethyl-ammonium bromide H_2SO_4 (CTAB) and 1N method (AOAC, 2005), method 973.18). The NDF content was determined by heat-stable α-amylase and sodium sulfite (Van Soest, Robertson, & Lewis, 1991). The amounts of Se concentrations in diets were measured by an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) device (using a Varian Model SpectrAA 220 atomic absorption spectrometer, Mulgrave, Victoria, Australia).

Blood samples were also collected from all the cows, -21, -10, 0, +10 and +21 days relative to calving. Blood samples were obtained by 20 ml vein puncture of the jugular vein during 4 h after morning feeding. After blood was collected, each sample was poured into tubes containing ethylene diamine tetraacetic acid (EDTA) for determining blood hematology parameters.

Table 1. Dry matter composition of basal diets fed to non-lactation or lactation cows.

	Dry cow - close up (%)	Fresh lactating dairy cow (%)
Corn silage	47.5	21.31
Alfalfa hay	12.5	17.04
Beet pulp	0	9.47
Cottonseed whole	0	4.26
Concentrate	40	47.92
Sum	100	100
Concentrate composition (%)		
Barely grain	25.2	24.523
Corn grain	28.74	29.67
Soybean seed whole heated	2.91	5.4
Fish meal	3.88	3.6
Soybean meal	4.85	17
Canola meal	13.57	0
Corn gluten meal	3.88	3.3
Cottonseed whole	5.98	9.5
Bentonite	1.03	0.7
Magnesium oxide	0.29	0.5
Biotin	0.02	0.01
Magnesium Sulphate	1.74	0
Calcium Carbonate	3.4	1.41
Calcium Chloride	1.45	0
Choline Chloride	0.83	0.45
Live yeast	0.01	0.007
Monensin	0.03	0.03
Avila 4	0.15	0.06
Niacin	0.1	0.1
Mineral premix[1]	0.97	0.5
Vitamin premix[2]	0.97	0.5
Sodium bicarbonate	0	1.2
Salt	0	0.5
Dicalcium phosphate	0	0.5
Potassium carbonate	0	0.4
B-complex vitamin premix	0	0.14
Sum	100	100
Calculated Composition		
Dry Matter (%)	41	59
NE_L (Mcal/kg DM)[3]	1.6	1.7
Protein (%)	13.7	16.1
NDF (%)	34.6	32.2
Ca (%)	1.31	0.94
P (%)	0.39	0.43
DCAD (meq kg^{-1})[3]	-57	288
Se (ppm)[4]	0.1	0.15

[1]Premix contained 50 g of Ca kg^{-1}, 11 g of Mg kg^{-1}, 15 g of Zn kg^{-1}, 3 g of Cu kg^{-1}, 0.15 g of I kg^{-1}, 0.05 g of Co kg^{-1}. [2]Premix contained 1800000 IU of vitamin kg^{-1}, 200000 IU of vitamin D kg^{-1}, 15000 IU of vitamin E kg^{-1} and 1.25 g of butylated hydroxytoluene kg^{-1} as a synthetic antioxidant. [3]Estimated from NRC (2001). [4]Selenium were measured by an Inductively Coupled Plasma-Mass Spectrometry.

Blood hematology parameters such as: including lymphocyte, red blood cells, hemoglobin and hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDWc) were evaluated by sysmex system (Sysmex K1000, TOA Ltd., Tokyo, Japan).

Measuring rectum temperature and vaginal discharge scores

The rectum temperature recorded for 10 days and measured by a thermometer during the morning feeding, and the recorded data were statistically analyzed.

Vaginal discharge scores measured at 10 and 21 days postpartum, the vaginal contents of the cows were extracted and graded from zero to 3 scores (Williams et al., 2005), as follows, and data were analyzed statistically.

Score 1: C (Clean mucous, lochia or no discharge)

Score 2: M (Mucopurulent)

Score 3: P (Purulent)

Reproduction data

Reproduction data the assessment of the reproductive status was carried out by rectal palpation, vaginal and ultrasonography examinations that were carried out from week two after parturition in weekly intervals to the confirmation of pregnancy. The following parameters were monitored: occurrence of clinical metabolic disorders (acidosis, ketosis, milk fever, retained placenta and displaced abomasum), clinical puerperal complications (mastitis and ovarian cyst) and distance to first estrus, insemination indexes and number of open days.

Statistical analysis

The data were analyzed by PROC MIXED of Statistical Analysis Software (SAS, 2004). Hematological blood parameters data (pre and postpartum) and average rectal temperature were analyzed as repeated measures and Time (DIM and week) was included in the model as a repeated variable. Based on the lowest Akaike information criterion, corrected Akaike information criterion, and Bayesian information criterion values for each variable analyzed the most suitable covariance structure were used (Littell, Henry, & Ammerman, 1998), 1998). The following model was used: $Y_{ijk} = \mu + T_i + Time_j + (T \times Time)_{ij} + Cow(i)_k + e_{ijk}$,

where $Y_{ijk}$is the dependent variable, μ is the overall mean, T_i is the fixed effect of treatment, $Time_j$ is the fixed effect of sampling time $(T \times Time)_{ij}$ is fixed interaction between treatment and sampling time, $Cow(i)_k$ is random effect of cow nested within treatment, and e_{ijk} is the error term. Previous lactation yield and the concentrations of hematological blood parameters obtained at −21 d relative to expected calving date were used as covariates and covariates were excluded from the model if they were not significant ($p > 0.1$). Data are reported as LSM and statistical significances were indicated at $p \leqslant 0.05$ and $0.05 < p \leq 0.10$ as trends toward significance using the Tukey's multiple comparison test.

Results and discussion

Hematological blood parameters

The results of blood tests showed that selenium supplementation had no significant effect on any hematological parameters of the blood during the transition period (Table 2 and 3). In the prepartum period, MCH tended to increase significantly in selenium methionine treatment ($p = 0.07$).

Table 2. Effect of different sources of selenium on the average hematological parameters of blood of dairy cows before parturition.

Trait	C	Se-S	Se-Y	Se-M	SEM	Treatment	Time	Treatment x Time
RBC (10^{-6} in microliter)	6.46	6.41	6.72	6.21	0.35	0.55	0.29	0.21
Hemoglobin (mg dL^{-1})	10.82	10.26	11.06	10.13	0.49	0.21	0.09	0.57
MCV (fl)	52.99	53.46	52.74	55.02	1.37	0.37	0.055	0.054
Lymphocyte (%)	55.99	58.38	60.38	67.54	6.6	0.36	0.63	0.49
RDWc (%)	17.82	17.75	18.16	17.62	1.39	0.98	0.35	0.93
MCH (pg)	16.71	16.11	16.84	17.34	0.44	0.07	0.001	0.55
MCHC (g dL^{-1})	31.51	30.5	31.86	32.28	0.81	0.21	0.0001<	0.28
Hematocrit (%)	34.91	34.1	35.27	33.38	1.94	0.76	0.04	0.65

a-b Means in the same column with no common superscripts are significantly different ($p < 0.05$).

Hematological analysis is a good way to detect metabolic disorders. It is also a useful way to diagnose diseases of some systems and organs. Although diagnosis of a disease can only be done based on the total number of complete red blood cells (CBCs), measurement of blood cells in the patient may provide valuable information for diagnosis (Roland, Drillich, & Iwersen, 2014). As with other species, a certain amount of physiological variability is observed in the hematological profile of the cattle, which depends on the age, sex, stress, ration, body condition score, production conditions, and animal activity (Wood, Mandell, & Swanson, 2010). White blood cells or leukocytes have an essential role in the immune system, and include subgroups of different neutrophils, eosinophils, granulocytes, monocytes and lymphocytes.

Research done to investigate the effect of selenium on hematological parameters has led to different results, which can be attributed to the length of the study period, the type of supplementation used, the type of livestock and the amount of selenium in the base diet.

Table 3. Effect of different sources of selenium on the average hematological parameters of blood of dairy cows after parturition.

Trait	C	Se-S	Se-Y	Se-M	SEM	Treatment	Time	Treatment × Time
RBC (106 in microliter)	6.13	6.17	6.58	6.26	0.32	0.49	0.06	0.25
Hemoglobin (mg dL-1)	10.41	10.12	11.35	10.29	0.45	0.058	0.14	0.41
MCV (fl)	53.38	53.74	52.94	53.36	1.66	0.97	0.09	0.56
Lymphocyte (%)	63.26	57.43	59.98	65.11	7.3	0.31	0.24	0.58
RDWc (%)	16.77	17.17	17.99	17.1	1.27	0.79	0.01	0.64
MCH (pg)	17.04	16.44	17.34	17.08	0.68	0.6	0.81	0.45
MCHC (g dL-1)	31.99	30.62	32.12	32.48	1.9	0.75	0.07	0.75
Hematocrit (%)	33.28	33.1	35.2	33.48	1.33	0.66	0.94	0.18

a-b Means in the same column with no common superscripts are significantly different ($p < 0.05$).

Shinde, Dass, and Garg (2009) consuming 0.3 ppm dry selenium in the form of sodium selenite in young buffalo rations, the researchers did not observe a change in the number of red blood cells, white blood cells, hematocrit, and hemoglobin concentration, which is consistent with the results of our study. Juniper, Phipps, Jones, and Bertin (2006) reported no significant difference in the number of red blood cells, white blood cells, hematocrit, and hemoglobin concentration in dairy cattle, broiler and sheep. There was no difference in hematological parameters in selenium fed lambs in the study of Mohri, Ehsani, Norouzian, Bami, and Seifi (2011). Faixova et al. (2007) observed that the number and resistance of red blood cells increased in lambs fed with diet containing 0.3 ppm selenium in dry matter in the form of selenium yeast.

Lymphocytes are produced in the lymphoid tissue and are dominated by white blood cells, but their ratio varies with age (Roland et al., 2014). In our study although lymphocyte percentage was not statistically significant, there was a significant difference between the treatments but the addition of selenium supplements was significantly higher in selenium methionine. White blood cells or leukocytes play a fundamental role in the defense system. Lymphocytosis can occur in the recovery phase of infectious diseases, and its reactive type is seen in acute infectious diseases, such as hepatitis, peritonitis, pericarditis, and mastitis (Gunter, Beck, & Phillips, 2003). The causes of lymphocytopenia include acute stress, bacterial infection, and reduced immunity. It also occurs in the event of destruction of lymph nodes (in tuberculosis, inflammation, and tumor incidence). The role of provoking selenium in immunity has already been proven (Mckenzie et al., 2003). Unfortunately, most studies have focused on the effects of selenium supplementation on selenium-deficient rations, and only limited reports of the effects of selenium sources on the safety performance of cows have been investigated. Selenium deficiency is an effective factor in reducing the fertility of lymphocytes and the transferrin receptor, which determines the proliferation of lymphocytes, reduces the number of lymphocytes in animals with selenium deficiency (Pighetti, Eskew, Reddy, & Sordillo, 1998). Researchers believe that selenium deficiency causes immunosuppression by preventing proliferation of lymphocytes (Sadeghian, Kojouri, & Mohebbi, 2012). In our study, with regard to the lack of difference between selenium-fed and control groups, it seems that the amount of selenium in the diet was sufficient to prevent the possible adverse effects of deficiency on white blood cells.

Rectal temperature and vaginal contents

The average rectal temperature in the experimental treatments is shown in Fig 1. The rectal temperature in the treatment containing selenium methionine was significantly ($p < 0.05$) lower than that of the control group. On the other hand, vaginal secretions were less in this group than in other groups (Tables 4 and 5).

Table 4. Status of the vaginal contents of the control and control cows in 10 days postpartum fed into organic and inorganic supplementation of selenium.

Experimental Treatments	Number	Clean mucous		Mucopurulent		Purulent	
		Ratio	percentage	Ratio	percentage	Ratio	percentage
C	6			6	(100)		
Se-S	6			5	(83.33)	1	(16.66)
Se-Y	6			5	(83.33)	1	(16.66)
Se-M	6	1	(16.66)	5	(83.33)		

Due to the low number of data, statistical analysis was not performed and only the number of cases was reported.

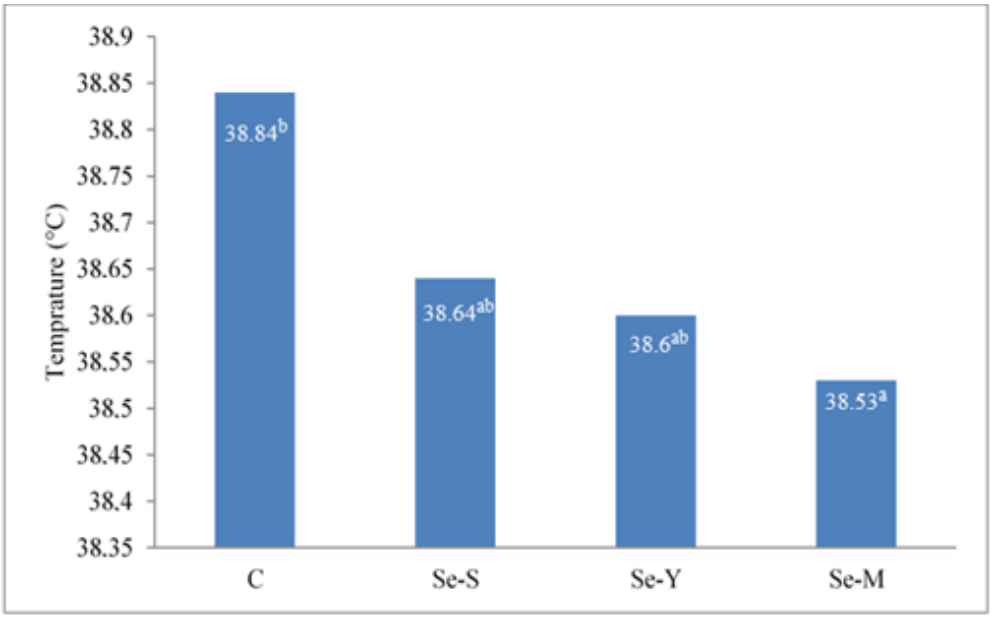

Figure 1. Average rectal temperature in different treatments.

Table 5. Status of the vaginal contents of the control and control cows in 21 days postpartum fed into organic and inorganic supplementation of selenium.

Experimental Treatments	Number	Clean mucous		Mucopurulent		Purulent	
		Ratio	percentage	Ratio	percentage	Ratio	percentage
C	6			1	(16.6)	5	(83.4)
Se-S	6	1	(16.6)	1	(16.6)	4	(66.6)
Se-Y	6			1	(16.6)	5	(83.4)
Se-M	6	3	(50)	2	(32.2)	1	(16.6)

Due to the low number of data, statistical analysis was not performed and only the number of cases was reported.

In our study, cows fed selenium supplementation showed less rectal temperature and purulent discharge. Selenium increases the immune function of dairy cattle and cows that have normal rectal temperature after delivery usually do not have inflammatory challenges after delivery and it may be associated with more feed intake, better milk production and enhanced immune system. In agreement with our results, Seboussi et al., (2016) significantly showed less purulent discharge in dairy cows supplemented with selenium yeast.

Reproductive parameters

The present study showed that addition of selenium supplement during transition period reduced distance to the first estrus, first insemination and distance from delivery to pregnancy and increase conception percent in the first insemination (Table 6).

Table 6. Average reproductive performance data in cows fed into organic and inorganic supplementation of selenium supplementation.

Reproductive parameters	C	Se-S	Se-Y	Se-M
Distance to first estrus (day)	51.75	48.66	45.66	40.8
Distance to first insemination (day)	62	60.83	58.83	60
Conception rate in first insemination (%)	25	50	83	80
Numbers of open days	149.25	130.33	100	93.4

Due to the low number of data, statistical analysis was not performed and only the number of cases was reported.

In addition, organic selenium supplements during the transition period have a positive influence on reproductive indices. In agreement with our research, other studies have shown the beneficial effects of selenium supplementation on pregnancy parameters in cattle and sheep (Gabryszuk & Klewiec, 2002; Sattar, Mirza, & Hussain, 2007). Contrary to the current research, Gunter et al. (2003) in cattle and Boland, Keane, Nowakowski, Brophy, and Crosby (2005) in sheep did not report any effect of selenium supplement on reproductive parameters.

In our study, selenium supplements (especially organic supplements) improve health status of dairy cows that prevent the increase of the rectal temperature, uterine disorders and retained placenta; so, improvements in reproductive parameters may be due to these reasons. Also uncontrolled or impaired immune and inflammatory responses in periparturient dairy cows are associated with increased incidence and severity of infectious diseases. The progressive development of oxidative stress during the transition from late gestation to peak lactation is thought to be a significant underlying factor leading to dysfunctional immune cell responses. Certain trace minerals, such as selenium, can ameliorate oxidative stress and reduce the severity of several economically important diseases in dairy cattle including mastitis and metritis. Many of the health benefits of Se can be attributed to the antioxidant functions of selenoproteins. Changes in selenoprotein activity as a consequence of Se nutritional status can directly alter a number of critical cellular functions involved in the inflammatory response. A better understanding of how selenium can optimize immune cell responses may facilitate the design of nutritional regimes that will reduce health disorders during the periparturient period (Sordillo, 2013).

Selenium and vitamin E as natural antioxidants have an important role in preventing the occurrence of retained placenta. These nutrients increase the activity of neutrophils, enhance their chemotactic effect and phagocytosis of opsonized pathogenic microorganisms. Oxidative stress may also contribute to placenta retaining. A great number of studies show that adequate supplement of selenium, zinc, cooper, iron and vitamins A, C and E playing a role of antioxidant; can reduce the percent of individuals with retained placenta. Adequately balanced rations with sufficient content of selenium, vitamin E and other antioxidants in food, appropriate housing of animals and good management lead to reducing the incidence of one of the most often ailments in dairy cows in postparturition period (Joksimović-Todorović & Davidović, 2013).

As shown in table 7, the use of selenium supplementation during the transition period did not affect the incidence of milk fever, ketosis, acidosis, displaced abomasum and ovarian cyst. While there was a lower incidence of retained placenta and mastitis in selenium-fed cows compared to the control group, there was no observation of retained placenta and mastitis in selenium methionine group. Similar to our study results, other studies have shown that selenium supplementation has reduced retained placenta (Joksimović-Todorović & Davidović, 2013; Yosathai, 2014) and mastitis (Mehdi & Dufrasne, 2016; Sordillo, 2013) in dairy cows.

Table 7. Incidence of clinical metabolic disorders and clinical puerperal complications in different treatments in cows fed into organic and inorganic supplementation of selenium.

Disorders	C	Se-S	Se-Y	Se-M
Milk fever	0	0	0	0
Retained placenta	2	1	1	0
Displaced abomasum	0	0	0	0
Acidosis	0	0	0	0
Ketosis	0	0	0	0
Mastitis	3	2	1	0
Ovarian cyst	0	0	0	0

Due to the low number of data, statistical analysis was not performed and only the number of cases was reported.

Conclusion

There was no significant difference in hematological parameters before and after delivery in experimental and control groups. However, in the prepartum period, MCH tended to increase significantly in selenium methionine treatment ($p < 0.05$). The mean of rectum temperature in the treatment of selenium methionine was significantly lower than that of the control group ($p < 0.05$). On the other hand, the purulent vaginal content, retained placenta, and mastitis were lower in this group. The results of this experiment showed that feeding organic selenium supplementation in multiparous dairy cow's diet may improve their health and reproduction.

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